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中国典型海区海洋古菌生态功能及环境适应机制的研究

Ecological Function and Environmental Adaptation Mechanisms of Pelagic Archaea in Typical Areas of China Seas

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摘 要

浮游古菌是海洋微型生物的重要组成之一。在真光层以下的深海大洋，海洋泉古菌Marine Group I（MGI）类群的生物量可占总原核生物量的20%。最近，环境基因组学研究的突破性进展和海洋泉古菌MGI类群纯培养株系的获得揭示出大部分MGI类群都是氨氧化古菌（Ammonia-Oxidizing Archaea, AOA）。由于MGI类群是海洋中数量最为丰富的微型生物之一，其必然在海洋碳、氮循环中起着重要作用，目前AOA已成为环境微生物生态学的研究热点之一。虽然针对浮游古菌尤其是MGI类群的生态调查表明该类群广泛分布于海洋水体中，但在中国海区，仅本课题组在2002年对东海长江口区域的浮游古菌进行了初步的多样性调查（Zeng et al., 2007）。对中国海区浮游古菌尤其是MGI类群的群落结构与生态功能的了解还十分有限。本论文通过分子生态学手段对中国海区浮游古菌开展了以下几方面的工作，并在对海洋泉古菌研究认知的基础上，进一步比较了湖泊泉古菌与海洋泉古菌环境适应机制的异同，拓展了对水环境常温古菌的认识：

1. 应用古菌16S rRNA基因调查了中国东海和南海六个站位表层水体中的古菌群落组成。研究结果表明河口区域的浮游古菌遗传多样性最高，其次是外海区域，而近海水体中的浮游古菌多样性最低。系统发育分析表明，中国海区的浮游古菌可分为泉古菌和广古菌：由于冲淡水的输入，河口的古菌群落由浮游古菌和陆源古菌组成；近岸和外海区域则没有受到陆源输入的影响。中国海区分布的优势浮游古菌类群在其他海区的相对丰度偏低，说明中国海区浮游古菌的地理分布模式有着一定的地域性特色。
2. 对南海11 m长沉积物柱状样中的古菌群落研究发现，分布于该沉积物的泉古菌和广古菌的主要系统发育类群是普遍分布于其他大洋沉积物的优势类群。基于RFLP带型的聚类分析和基于系统发育类群的RDA分析表明该沉积物中的古菌群落组成有深度分布趋势。古菌功能基因PCR筛选表明，表层沉积物中的MGI类群含有氨单加氧酶α亚基编码基因—*amoA*基因。由于本课题组曾在同一样品检测到厌氧氨氧化细菌的16S rRNA基因序列和氨氧化细菌的16S rRNA基因序列，说明古菌与其他N循环相关的微型生物一起参与了南海沉积

物的N循环过程。

3. 结合古菌16S rRNA基因、泉古菌氨氧化基因*amoA*和自养固碳基因*accA*研究了南海三个远洋站位全水柱剖面的古菌群落结构与生态功能。在不同站位的相同水系中，古菌16S rRNA基因、*amoA*和*accA*基因的丰度量级和遗传多样性都较为相似；而在垂直梯度上，深层水体（> 1000 m）中的古菌基因丰度要显著低于上层（< 200 m）和中层水体（200 m - 1000 m）中的古菌基因丰度（除了古菌*accA*基因在上层水体中极为稀少）。古菌16S rRNA基因DGGE分析和功能基因克隆文库结果表明上层水体与中、深层水体的古菌种群存在明显的进化分歧。统计分析不同基因间的丰度比表明，上层水体中的MGI类群缺少自养固碳基因，深层水体的MGI类群缺少氨氧化基因。这些结果预示了浮游泉古菌在大洋不同水体有着种群演替和生态功能的变迁。
4. 结合泉古菌16S rRNA基因、*amoA*和*accA*基因研究了东海黑潮区域两个站位真光层和弱光层上层水体中的泉古菌生态位分化现象及环境适应机制。与我们在南海的研究结果相似，东海上层水体中的大部分MGI类群含有*amoA*基因，但缺少*accA*基因，说明上层大洋中的浮游泉古菌可能是以化能无机异养方式生存。生态型模拟计算表明，上层水体中的浮游泉古菌同样分化为适应不同深度梯度的生态型，说明光可能是造成这种生态位分化的重要因子。
5. 通过泉古菌*amoA*基因PCR—克隆文库方法比较了五个青藏湖泊的氨氧化微生物的群落结构。系统发育分析和统计分析表明湖泊之间的氨氧化古菌群落结构受盐度调控，而不同湖泊中的氨氧化细菌则隶属于*Nitrosomonas*属的一个单一的系统发育分支。与海洋浮游泉古菌在深度梯度上的生态位分化相反，湖泊内部不同水层的氨氧化古菌趋同进化，说明湖泊和海洋中的泉古菌在环境适应机制上存在着差别。

关键词：浮游古菌；泉古菌；氨氧化古菌；16S rRNA 基因；*amoA* 基因；*accA* 基因自养固碳；系统发育分析；生态功能；环境适应机制；中国海；青藏高原湖泊

Abstract

Planktonic archaea are one of the most important components of Marine microbes. Marine *Crenarchaeota* Group I (MGI), one phylogenetic group of the planktonic archaea, are typically relatively more abundant in the dark ocean and are thought to account for 20% of all prokaryotic cells in the global ocean. Recently, the great breakthrough in studies of metagenomics and pure culture of MGI revealed that functional group of them—ammonia-oxidizing archaea (AOA). Given the suggestion abundance of MGI in the marine environments, AOA would play an important role in Earth's biogeochemistry and have become a new hotspot in environmental microbial ecology. Although numerous studies have demonstrated that MGI are one of the most abundant and ubiquitous life forms in the ocean, reports about diversity and distribution of planktonic archaea in China Seas are very rare. Zeng et al. (2007) have studied the genetic diversity of planktonic archaea in the Yangtze River estuary. However, the community structure and ecological function of planktonic archaea in China Seas is still unclear. Here we investigated the geographic distribution, genetic diversity, ecological function and environmental adaptation mechanisms of planktonic archaea in typical areas of China Seas and Tibetan lakes by using molecular ecological methods.

1. The community structures of planktonic archaea were investigated for the surface water from six stations in the East (ECS) and South China Seas (SCS) by using 16S rRNA gene sequence analysis. The results demonstrated that the patterns of archaeal diversity in China Seas with the highest diversity in estuarine regions, moderate diversity in offshore regions and the lowest diversity in inshore regions. Phylogenetic analyses indicated that all archaeal 16S rRNA gene sequences obtained from China Seas belonged to the phyla *Crenarchaeota* and *Euryarchaeota*. In the estuarine areas, some archaeal clones were non-marine planktonic archaea, probably of terrestrial origin; while planktonic archaea dominated in inshore and offshore regions. Moreover, the dominant archaeal species were different from previous studies in other oceans of the world, revealing local specific characteristics of archaeal diversity.
2. The community structures of archaea were determined for an 11 m-long

subseafloor sediment core from the SCS. Our results revealed that the dominant phylogenetic groups of *Crenarchaeota* and *Euryarchaeota* are ubiquitous in pelagic sediments. RFLP types based clustering analysis and RDA analysis revealed depth-related changes in the community compositions of archaea in this sediment. Based on PCR screening of the functional genes of archaea, archaeal *amoA* genes were present in the surface layer of this sediment. Since our lab have recovered some 16S rRNA gene sequences of anammox- and ammonia-oxidizing bacteria from the same sample, we concluded that MGI *Crenarchaeota* together with other nitrifying microbes play important roles in the N cycling in the SCS sediments.

3. The community structure and ecological function of planktonic archaea were investigated for three pelagic stations in the SCS by using a combination of archaeal 16S rRNA, *amoA* and *accA* gene sequence analysis. Our results indicated that the abundance and genetic diversity of these genes were similar among the three stations. However, they are vertically stratified and significantly less abundant in the bathypelagia (< 1000 m) than in the epi- and mesopelagia at each station, except that archaeal *accA* genes were almost absent in the epipelagia. Furthermore, ratios of the archaeal *amoA* gene to 16S rRNA gene abundance slightly decreased from the epi- to bathypelagia, whereas the ratios of archaeal *accA* gene to MGI 16S rRNA gene or to archaeal *amoA* gene abundance increased with depth, suggesting that the trophic strategy of *Crenarchaeota* may change from the epi- to meso- or bathypelagia. Overall, our results show strong depth partitioning of crenarchaeal populations in the SCS and suggest a shift in their physiology and ecological function with increasing depth.
4. We studied the niche-partitioning and environmental adaptation mechanisms of marine *Crenarchaeota* in two pelagic stations of the ECS by using a combination of archaeal 16S rRNA, *amoA* and *accA* gene sequence analysis. Consistent with the results of our previous SCS investigation, we found most if not all *Crenarchaeota* contained *amoA* gene, but lack of *accA* gene, suggesting marine *Crenarchaeota* may live by chemolithoheterotrophy in the upper ocean. The results of ecotype stimulation showed that marine *Crenarchaeota* divided into several ecotypes to adapt local environments in the vertical scale of the upper ocean, implying that light may play an important role in controlling the niche-partitioning of marine *Crenarchaeota*.

5. Community structures of planktonic AOA and ammonia-oxidizing bacteria (AOB) were investigated for five high-altitude Tibetan lakes. Phylogenetic and statistical analyses indicated that a heterogeneous distribution of the AOA communities among lakes largely caused by salinity, whereas AOB communities were dominated by a unique monophylogenetic lineage within *Nitrosomonas*. Unlike depth-stratified community of marine *Crenarchaeota*, the uniform chemical properties within Tibetan lakes and their geographical isolation may favour relatively homogeneous AOA communities within lakes.

Keywords: planktonic archaea; *Crenarchaeota*; ammnia-oxidizing archaea; 16S rRNA; *amoA*; *accA*; autotrophic carbon fixation; phylogenetic analysis; ecological function; environmental adaptation mechanisms; China Seas; Tibetan lakes